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(21) International Application Number: PCT/GB90/01459 (22) International Filing Date: 21 September 1990 (21.09.90) (30) Priority data: 8921470.4 22 September 1989 (22.09.89) GB (71) Applicant (for all designated States except US): PEPTIDE TECHNOLOGY LIMITED [AU/AU]; 4-10 Inman Road, P.O. Box 444, Dee Why, NSW 2099 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only) : MOSS, Bernard, Anthony [GB/AU]; 153 Riverview Street, Lane Cove, NSW 2066 (AU). ASTON, Roger [GB/AU]; Lot 2430 Highs Road, West Pennant Hills, NSW 2120 (AU). COWDEN, William, Bulter [US/AU]; 56 Uranby Village, Crozier Circuit, Kambah, ACT 2902 (AU).		(74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US. Published <i>With international search report.</i>
(54) Title: VACCINES (57) Abstract <p>Solid vaccine compositions comprise an antigenic substance, a saponin and a polycationic adjuvant such as DEAE-dextran. The antigenic substance gives rise to antibodies either for the purpose of fighting infections or for other purposes: for example, antibodies against GnRH can modulate fertility. The combination of a saponin and a polycationic adjuvant gives the vaccine improved longevity and enables it to be used as an implant.</p>		

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VACCINES

This invention relates to vaccines.

Vaccines have classically been used in the prevention of disease. An antigen having antigenic characteristics of a disease-causing entity (such as a microbe or toxin) is parenterally administered to man or another animal, and the animal's immune system is stimulated to produce antibodies which will react both with the antigen administered and the disease-causing agent itself.

More recently, vaccines have also been used for other purposes, particularly in the modulation of hormonal activity. Antibodies generated against a hormone antigen may cross react with endogenous hormone in the animal's body. A primary (but not exclusive) application of this new vaccine technology is the production of vaccines for fertility control.

The antigenicity of many potential antigens is frequently enhanced by the co-application of antigens with immunoadjuvants, which may be regarded as substances which, while not necessarily being antigenic themselves, potentiate or enhance an animal's immune response to the challenging antigen.

A wide range of adjuvants is known. Examples include Freund's complete and incomplete adjuvants (FCA and FIA), saponins, aluminium compounds, including aluminium phosphate and aluminium hydroxide (particularly in the form known as alhydrogel),

1 polycationic electrolytes, polyanionic electrolytes,
2 muramyl dipeptide and Adjuvant 65, which contains
3 highly refined peanut oil and chemically pure mannide
4 monooleate and aluminium monostearate as emulsifier and
5 stabiliser respectively.

6
7 Even with the availability of the above and many other
8 adjuvants, it is sometimes difficult to formulate
9 vaccines for inducing antibodies against particular
10 antigens. Gonadotrophin releasing hormone (GnRH,
11 otherwise known as luteinising hormone releasing
12 hormone (LHRH) is a case in point.

13
14 It is commercially desirable to formulate a GnRH
15 vaccine for veterinary use, particularly but not
16 exclusively for domestic livestock. An antigen GnRH
17 preparation is useful as a fertility regulating or an
18 immunological neutering vaccine in male (for
19 immunocastration) and female (for immunospaying)
20 animals. It is indicative of the difficulties of
21 formulating a GnRH vaccine that the neutering
22 properties of GnRH have been known since 1972, but it
23 is only now that vaccines based on GnRH are beginning
24 to emerge commercially [Hoskinson et al, Aust. J.
25 Biotech, 4, 166-170(1990)]. The utility of a GnRH
26 vaccine is demonstrated by the experiences of
27 Australian stock farmers. In extensively grazed
28 cattle raised for beef, up to 80% of the cull cows can
29 become pregnant, thereby causing the farmer
30 considerable economic loss at slaughter because the
31 carcase value is downgraded.

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1 GnRH can be formulated as a vaccine with Freund's
2 complete adjuvant (FCA), which comprises a suspension
3 of heat-killed M. tuberculosis mycobacteria in mineral
4 oil containing a surfactant. Although FCA is
5 recognised as a powerful adjuvant, it has not found
6 wide application outside the laboratory because of the
7 adverse tissue reaction it provokes in recipient
8 animals. In fact, FCA is banned from veterinary use.

9
10 A different approach to the problem is disclosed in
11 WO-A-8706129, which suggests the use of an implant
12 containing microencapsulated immunogens of GnRH (or
13 another antigen) within a biodegradable polymer. The
14 level of development of this technology as a practical
15 matter, is still unclear; however, no commercial
16 product based on the technology appears yet to have
17 been launched.

18
19 The only GnRH vaccine on the market is a two-shot
20 mineral oil based emulsion vaccine in accordance with
21 the teaching of WO-A-8801177 [Hoskinson et al, Aust J.
22 Biotech, 4 166-170 (1990)]. Although excellent results
23 can be obtained by the use of such a vaccine, it would
24 be desirable to eliminate the necessity of having oil
25 present, and it would also be desirable to improve the
26 longevity of action of the vaccine so that two shots
27 were not required. The problem with having the mineral
28 oil present, is that it can cause localised irritation
29 at the site of injection or implantation, leading among
30 other undesirable effects, to the formation of sterile
31 abscesses and granulomas; further, it is generally
32 desirable to avoid the use of petrochemical-derived

1 materials in preparations administered to animals,
2 particularly parenterally.

3

4 The problem with a two-shot vaccine is more of a
5 practical one for the farmer. The farmer will want to
6 muster his livestock once a year in order to tag the
7 herd and also for other veterinary purposes. The
8 vaccine can therefore be conveniently administered at
9 the mustering. However, if a second muster is needed
10 several weeks later for a second, booster vaccination,
11 this represents a considerable expenditure of effort
12 purely for vaccination purposes, as there is otherwise
13 no need for the second muster. In pastoral regions
14 where ovine footrot is a problem, there is a need for
15 two or more booster vaccinations to maintain high
16 antibody levels in the sheep during the critical
17 season. Longevity of action is therefore a desirable
18 goal for a vaccine in order to avoid the unnecessary
19 handling of animals.

20

21 It can be seen that there is a need for a vaccine which
22 at least partially solves one or both of the two
23 problems discussed above. Furthermore, it would be
24 preferred if the action of the vaccine was reversible,
25 particularly for a fertility-regulating vaccine such as
26 one based on GnRH, so as to widen the potential market
27 for the vaccine, for example to include horses.
28 Further, it would be preferred if an effective vaccine
29 could be formulated in solid form, which resulted in
30 minimal tissue reaction at the implantation site and
31 which conferred user safety by minimising the
32 possibility of a farmer injecting himself with the

33

1 formulation and was able to provide improved shelf life
2 stability.

3

4 According to a first aspect of the present invention,
5 there is provided a solid vaccine composition
6 comprising an antigenic substance capable of inducing
7 the generation of antibodies on parenteral
8 administration to an animal, a saponin and a
9 polycationic adjuvant.

10

11 Although saponin and polycationic compounds have
12 individually been used as adjuvants in the past, as
13 have many other adjuvants, the art does not seem to
14 have realised that this particular combination of
15 adjuvants, when formulated as a solid, has particularly
16 beneficial properties when used in a vaccine in
17 accordance with this invention.

18

19 In the art, Solyom (Dev. Biol. Stand 34 169-178 (1977))
20 has separately evaluated DEAE-dextran (a polycationic
21 adjuvant) and saponin in foot and mouth disease
22 vaccines. Mitev et al (Vet. Med. Nauki, 12 16-22
23 (1975)) teaches that vaccines containing DEAE-dextran
24 are generally inferior to oil-based vaccines; it is
25 also suggested that saponin is a better sole adjuvant
26 than DEAE-dextran. Gorskii (Uchenye Zap. Kazans. Vet.
27 Inst. 122 48-49 (1976)) takes the opposite view to
28 Mitev et al and teaches that DEAE-dextran is a superior
29 adjuvant to saponin for foot and mouth disease virus.
30 The efficacy of saponin, DEAE-dextran and aluminium
31 hydroxide in a foot and mouth disease vaccine have also
32 been evaluated in pig trials; here, DEAE-dextran
33 performed better than $Al(OH)_3$ or saponin (Sellers and

1 Herniman Brit. Vet. J. 30 440-445 (1974)). The short
2 lived nature f the immune response elicited to foot
3 and mouth disease by DEAE-dextran or saponin has been
4 described by Anderson et al (Res. in Vet. Sci. 12
5 351-357 (1971)). In contrast, this group demonstrate
6 that oil-based emulsion adjuvants have longevity. The
7 superior efficacy of Freund's adjuvant to others such
8 as DEAE-dextran is described by Beh and Lascelles
9 (Immunology 54 487-495 (1985)). Indeed, these authors
10 state that no interactions between the different
11 classes of adjuvant examined is observed. WO-A-8801177
12 teaches synergy between an oil adjuvant and a
13 polycationic adjuvant; although this formulation is
14 efficacious with GnRH and exhibits longevity, it relies
15 on the presence of an oil-based emulsion; and the
16 present invention avoids the use of oil. This type of
17 synergy (where the immune response exceeds the sum of
18 the immune responses of the individual components) is
19 also observed by using dextran sulphate (a polyanionic
20 adjuvant) in conjunction with saponin, Vanselow et al
21 (Vet. Rec. 117 37-43 (1985)). WO 88/07547 teaches that
22 the combination of DEAE-dextran and saponin in solution
23 is useful at eliciting antibody when mixed with
24 antigen; however it is known that such combinations, or
25 the use of these adjuvants singly in solution, results
26 in a short-lived immune response of little or no
27 practical veterinary value. In contrast, the
28 formulation of these adjuvants into a solid implant
29 vaccine by the particular methods described here
30 provides the basis for veterinary vaccines with
31 longevity.

32

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1 In a vaccine in accordance with th present invention,
2 the antigenic substance may give rise to antibodies
3 against a disease-causing agent, or against an agent
4 (such as a hormone) which does not normally give rise
5 to a disease. The disease causing agent may be a
6 structural component or toxin of a virus, bacterium or
7 other microbe. Examples of virally-caused diseases
8 which may be controlled by means of the present
9 invention include foot and mouth disease (FMD),
10 infectious bursal disease (IBD), Newcastle disease,
11 rabies, egg drop syndrome virus (EDS₇₆) disease in
12 poultry, calcivirus, rhinotracheitis in cattle, bovine
13 ephemeral fever (BEF) and respiratory virus, among
14 others.

15 Examples of bacterially-caused diseases include
16 botulism, clostridial infections, foot rot (for a
17 vaccine against which the antigenic substance may
18 comprise Bacterioides nodusus recombinant pili),
19 Caseous Lymphadenitis CLA in sheep caused by
20 Corynebacterium pseudotuberculosis toxin, among others.
21 Other microbial, such as fungal or protozoal,
22 infections may also be controlled by means of the
23 present invention.

24

25 Of the vaccines in accordance with this invention which
26 caused the generation of antibodies against
27 non-disease-causing agents, a vaccine against GnRH is
28 one of the most preferred. Vaccines against other
29 peptide hormones (for example growth hormone) are also
30 commercially significant as are vaccines against
31 certain non-peptide hormones, for example steroid
32 hormones.

33

1 The antigenic substance may consist of the entity
2 against which antibodies are to be raised. This may
3 frequently be the case when the antigenic substance is
4 characteristic of a disease-causing agent. However, in
5 some cases (particularly but not exclusively those
6 cases where it is desired to raise antibodies against
7 non-disease-causing agents), the antigenic substance
8 may comprise a target antigenic moiety conjugated to a
9 carrier. The carrier will generally be selected so as
10 not to be recognised as "self" by the animal to which
11 the vaccine is to be administered. Suitable carriers
12 include albumins including ovalbumin (not for poultry),
13 bovine serum albumin (not for cattle), human serum
14 albumin (not for humans) and other albumins.
15 Alternatively, the carrier may be a different protein
16 or other molecule. Examples of proteinaceous carriers
17 other than albumin include keyhole limpet haemocyanin
18 and beta-galactosidase, among others. It is not
19 necessary for the carrier either to be a protein or
20 even proteinaceous, but such carriers are preferred.
21 Carriers may in general be available from Sigma, Pierce
22 or Bio Rad, or any other convenient supplier.

23
24 The nature of the implant vaccine described here also
25 lends itself to the use of several antigens either
26 linked to the same or different carriers. Similarly,
27 in cases where immunological problems such as antigen
28 competition occur or when one antigen preparation
29 inactivates another via mixing, the implant vaccine may
30 be formulated so that different antigens are presented
31 in distinct implants keeping individual antigens
32 separate.

33

1 Th target antigenic moi ty may be conjugated to the
2 carrier, when a carrier is used, by any convenient
3 means. Suitable conjugators include glutaraldehyde,
4 toluene diisocyanate, carbodiimide, or any other
5 suitable conjugator, which may effect a linkage through
6 a carboxyamino group. Such groups may be created by
7 means of activated diacid, such as an acid dichloride
8 or an acid anhydride. Disuccinimidyl compounds are
9 particularly suitable, especially disuccinimidyl
10 tartrate and disuccinimidyl suberate, both of which are
11 available from Pierce, as are many of the other
12 conjugators that are preferred for use in this
13 invention. Other acceptable conjugators effect a
14 linkage through thiol groups as disulphides or
15 thioethers; suitable conjugators include SPDP and other
16 aminodisulphydril cross-linkers and double agents such
17 as MBS.

18
19 The amount of antigenic substance present in each
20 vaccine dose will of course depend on the identity of
21 the antigenic substance and whether it is conjugated
22 with a carrier. Typically, for a conjugate vaccine it
23 may be expected that the amount of material
24 administered per injection should be from 10 μ g to 10mg.
25 For example in a GnRH vaccine, 2mg of conjugates may be
26 present of which 100 to 800 μ g would be GnRH (typically
27 200 μ g of GnRH) and 1.9 to 1.2mg would be carrier.
28 These amounts are purely illustrative and indicate
29 suitable levels for GnRH vaccines.

30
31 The saponin may be obtained from any convenient source.
32 Saponin is available from Sigma Chemical Co, USA, and a
33 particularly purified and lyophilised form is available

1 from Superfos Biosector A/S, Denmark, under the trade
2 mark QUIL-A. It should be noted that it is not a
3 prerequisite that a single species be used; mixtures of
4 different saponins are quite acceptable. Preferred
5 saponins include those disclosed in WO-A-8809336.

6
7 The amount of saponin present can be any appropriate
8 amount. Amounts of from 50 μ g to 50mg may be suitable,
9 for example, from 500 μ g to 5mg; an amount of about 1mg
10 may be found to be particularly appropriate.

11
12 The polycationic adjuvant may be any suitable such
13 adjuvant, particularly including those disclosed in
14 WO-A-8801177. Diethylaminoethyl dextran (DEAE-dextran)
15 is particularly useful and may be supplied as the free
16 base or the hydrochloride or any other appropriate acid
17 addition salt. Other suitable polycationic adjuvants
18 include polylysine, polyethyleneimine and chitosan,
19 which again may be supplied either as the free base or
20 as an acid addition salt. The polycationic adjuvant
21 may be buffered to be at or near physiological pH, as
22 will subsequently be described.

23
24 It should be noted that the invention contemplates the
25 use of a conjugate of the antigenic substance and
26 polycationic adjuvant as well as mere mixtures of two
27 separate components. The antigenic moiety and
28 polycationic moiety may therefore be covalently
29 attached, either directly or by means of a linking
30 element.

31
32 A vaccine in accordance with the invention can
33 optionally contain certain other components. In

1 particular, the vaccine may contain a filler. The most
2 preferred filler is calcium phosphate, particularly
3 dibasic calcium phosphate dihydrate. A particularly
4 suitable form of dibasic calcium phosphate dihydrate is
5 sold under the trade mark EMCOMPRESS by Edward Mendell
6 Co. Inc., Carmel, New York, USA. This preparation
7 conforms to USP XX/FCC III. The average particle size
8 of the calcium phosphate (or any other filler) may
9 range from 20 to 200 μ m, with 50 to 150 μ m being a
10 typical range. Average particle sizes of about 100 μ m
11 are common. Alternative fillers may also be in the
12 form of biodegradable polymers (see later).

13

14 The amount of calcium phosphate or equivalent filler
15 may be such as to adjust the volume of the vaccine
16 composition to a convenient amount. For example, a
17 convenient maximum volume might be 1ml, but the
18 circumstances will vary from case to case. The amount
19 of calcium phosphate (or total filler) per unit dose
20 vaccine formulation may range from 10mg to 1g, with
21 from 20mg to 200mg being typical. The filler may
22 comprise from 5 to 95% w/w of the weight of the
23 formulation, with from 30 to 80% w/w being typical.

24

25 A further filler, which may for example be used in
26 conjunction with the preferred calcium phosphate
27 described above, is lactose. A suitable source of
28 anhydrous lactose is direct compression lactose, such
29 as that sold under the trade mark DCLactose 21 by
30 De Melkindustrie Veghel BV of Veghel, The Netherlands.
31 This formulation of anhydrous lactose satisfies the
32 requirements of USP XXI/NF XVI. The amount of lactose

33

1 present can vary from 0 to 15% w/w, for example from 5
2 to 10% w/w, based on the total weight of the vaccine
3 formulation.

4

5 Another filler which may be used is cholesterol. A
6 suitable source is the USP grade from Croda Inc, USA.
7 The amount of cholesterol present may vary from 0 to
8 80% w/w, for example from 25 to 50% w/w, based on the
9 total weight of the vaccine formulation.

10

11 Other (generally dry) fillers may be present, for
12*^p+91Xexample, sodium calcium hypophosphate or dry (for
13 example freeze dried) aluminium hydroxide may be used
14 as a filler.

15

16 Because preferred formulations of vaccines in
17 accordance with the invention include tablets and
18 extrusions, the presence of a lubricant to aid in
19 formulation is desirable. Any suitable lubricant, such
20 as magnesium stearate, can be used, but it is generally
21 preferred for the lubricant to comprise a hydrogenated
22 vegetable oil, such as that sold under the trade mark
23 LUBRITAB by Edward Mendell Co, Inc, Carmel, New York,
24 USA.

25

26 The lubricant may be present in an amount up to 5% w/w,
27 based on the total weight of the vaccine formulation,
28 but is generally present in a range of from 0.5 to 2.5%
29 w/w.

30

31 Other adjuvants or components which stimulate the
32 immune response may be present in vaccine formulations
33 in accordance with the invention, if desired. For

1 example, muramyl dipeptid may be present. Lipid-based
2 products may also be present for this purpose.

3

4 A buffer may be present, for example to counteract the
5 effect that the polycationic adjuvant has on the pH
6 when the vaccine is administered.

7

8 Other acceptable excipients can be present in the
9 vaccine formulation in suitable amounts. It is
10 however, not necessary for any other ingredients to be
11 present.

12

13 The vaccines in accordance with the invention are solid
14 and may therefore be in the form of a powder or
15 granules, either of which may optionally be
16 encapsulated, or compressed or otherwise prepared to
17 form a tablet, bolus or extruded strip which may be cut
18 or otherwise post-formed to any convenient length
19 and/or shape.

20

21 In view of the generally solid nature of vaccines in
22 accordance with the invention, they will generally be
23 dry. This is not to mean that the vaccine as a whole,
24 or any of the ingredients, is necessarily anhydrous.

25

26 Vaccines in accordance with the invention may be
27 implantable and/or injectable, and will therefore for
28 preference be sterile. A subcutaneously implantable
29 vaccine is preferred, but an intramuscularly
30 implantable vaccine is also viable. Intraperitoneally
31 implantable vaccines are less preferred but may be
32 suitable in some circumstances. It will not generally
33 be appropriate to implant or inject vaccines in

1 accordance with the invention intravenously, as
2 saponins have a powerful lytic effect on r d blood
3 cells.

4
5 Although there may be some applications in which the
6 present invention is suitable for treating humans,
7 species of animals which can usefully be treated by
8 means of the present invention include cattle, pigs,
9 sheep, deer, camels, horses, dogs and cats, to give but
10 a few examples. In each of these and other species the
11 vaccines of the invention can be used for conventional
12 purposes for the treatment of disease. In addition, in
13 each of these and other species, vaccines in accordance
14 with the invention can be used for purposes other than
15 preventing disease, for example for modulating hormone
16 activity, particularly fertility hormone activity. In
17 cattle, vaccines in accordance with the invention may
18 be used bio-chemically to immunologically neuter bulls
19 and cows. Immunoneutering of sheep and pigs is also a
20 particularly preferred application. Immunocastration
21 of ram lambs destined for the prime lamb market is a
22 specific example.

23
24 It is by no means necessary for vaccines in accordance
25 with the invention to be restricted to having a single
26 function. Disease-preventing vaccines may be
27 multifunctional, as may hormone activity-modulating
28 vaccines. Additionally, vaccines in accordance with
29 the invention can combine very different activities,
30 such as disease prevention and hormone activity
31 regulation.

32
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1 Vaccines in accordance with the invention can be
2 prepared by any convenient method, all of which are
3 within the scope of the invention. It may be
4 appropriate under some circumstances to prepare
5 vaccines merely by adequately admixing the ingredients.
6 According to a second aspect of the invention,
7 therefore, there is provided a process for the
8 preparation of a vaccine, the process comprising
9 admixing (a) an antigenic substance capable of inducing
10 the generation of antibodies on parenteral
11 administration to an animal, (b) a saponin and (c) a
12 polycationic adjuvant.

13

14 A particularly preferred way to prepare a vaccine in
15 accordance with the first aspect of the invention
16 involves freeze drying the components from a (for
17 example aqueous) solution. For some reason that is not
18 entirely clear, but may be to do with the degree of
19 intimate admixture obtainable by such a process,
20 vaccines prepared in this method have been found to be
21 very satisfactory.

22

23 According to a third aspect of the present invention,
24 therefore, there is provided a process for the
25 preparation of a vaccine, the process comprising
26 lyophilising a solution (for example an aqueous
27 solution) of (a) an antigenic substance capable of
28 inducing the generation of antibodies on parenteral
29 administration to an animal, (b) a saponin and (c) a
30 polycationic adjuvant.

31

32

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1 The solution is preferably stirred thoroughly (for
2 example, for at least 2 hours or even 24 hours or more)
3 prior to lyophilisation for optimum results.

4

5 The solution will generally be aqueous and may include
6 a buffer to bring the pH of the solution near to
7 neutrality and/or physiological pH.

8

9 In certain cases (for example to prolong the release of
10 active vaccine constituent) it may be preferred to
11 admix the antigenic substance and the two adjuvants
12 with the fillers by wet granulation and lyophilise the
13 common mixture.

14

15 Although under some circumstances, as discussed above,
16 the antigenic substance and the two adjuvants (the
17 saponin and the polycationic adjuvant) can be
18 lyophilised from a common solution, it may under some
19 circumstances be possible to prepare satisfactorily an
20 immunoadjuvant composition, to which the antigenic
21 substance can subsequently be added.

22

23 According to a fourth aspect of the present invention,
24 therefore, there is provided an immunoadjuvant
25 comprising a saponin and a polycationic adjuvant.

26

27 As discussed above, vaccines in accordance with the
28 invention are preferably solid. The vaccine may for
29 preference be in tablet form or be formed by extrusion
30 to a desired length. A vaccine including its active
31 components in accordance with the invention may be
32 coated. The coat may be water impermeable but
33 erodible, so that after a suitable period of time the

1 coat will dissolve or otherwise break down to enable
2 release of the active components of the vaccine. It is
3 possible in this way to provide a plurality of
4 implants, ranging from being non-coated to each having
5 a coat of particular thickness and/or erodibility
6 characteristics such that, for example, one implant
7 might release active components immediately to provide
8 a primary sensitising dose while others may release
9 weeks or even months later to provide boosting doses
10 and thereby extend the longevity of the immune
11 response.

12

13 A variety of materials can be used for the coat,
14 whether as an erodible or biodegradable coat.
15 Polyesters constitute a preferred category of
16 erodible/biodegradable encapsulating polymers that are
17 also biocompatible; examples include polylactide,
18 polyglycolide and poly(lactide-co-glycolide) such as
19 those sold under the trade mark MEDISORB by the Dupont
20 Company, USA., poly(hydroxybutyric acid) such as that
21 sold by Chemie Holding, Linz, Austria,
22 poly(hydroxybutyric acid-co-valeric acid) such as that
23 sold by Aldrich Chemicals, USA, or ICI, UK. Other
24 suitable erodible biodegradable polymers include
25 polyacetals, polyorthoesters and polyorthocarbonates
26 as is disclosed in EP-A-0052510 (Syntex). It will be
27 appreciated that coatings can conveniently be made from
28 a mixture of the above or other polymers, particularly
29 when ester derivatives are used.

30

31 The coat may alternatively remain essentially intact
32 after implantation; it may be semi-permeable to ensure
33 adequate leaching out of ingredient. The coat may be

1 non-biodegradable if desired. Cellulose derivatives
2 constitute a suitable category of polymer; examples
3 include ethyl cellulose, such as that sold under the
4 trade mark ETHOCELL by Dow Chemical Co, USA, methyl
5 cellulose, such as that sold under the trade mark
6 METHOCELL by Dow Chemical Co, USA and
7 hydroxypropylmethyl cellulose, such as that sold under
8 the trade mark PHARMACOAT by Shinetsu Chemical Co of
9 Japan. Methacrylate derivatives form another suitable
10 class. Examples include a 1:2 poly (methacrylic acid,
11 methylmethacrylate) polymer sold under the trade mark
12 EUDRAGIT S100 by Rohm Pharma, West Germany and 1:2:1
13 poly (butylmethacrylate, methacrylate,
14 methylmethacrylate) polymer sold under the trade mark
15 EUDRAGIT E100 also by Rohm Pharma.

16

17 It should be noted that the invention in certain
18 circumstances (for example to allow enable pulsed
19 antigen/adjuvant release at delayed time intervals)
20 contemplates coating granules of the active
21 antigen/adjuvant mix itself by solvent evaporation onto
22 granules, wet granulation or fluidised bed spray
23 coating or other means, with a mixture of the above or
24 other erodible or biodegradable polymers prior to
25 formulating into a vaccine as granulates or as
26 compressed tablets. Such polymer coated granules are
27 particularly useful as vaccine implants when used in
28 conjunction with cholesterol as a filler.

29

30 According to a fifth aspect of the invention, there is
31 provided a method of treating a human or another
32 animal, the method comprising administering a vaccine
33 in accordance with the first aspect of the invention.

1
2 The invention therefor encompasses the use of (a) an
3 antigenic substance capable of inducing the generation
4 of antibodies on parenteral administration to an
5 animal, (b) a saponin and (c) a polycationic adjuvant
6 in the preparation of a vaccine.

7
8 As vaccines in accordance with the first aspect of the
9 invention can be used as one-shot vaccines, a single
10 shot constitutes the preferred treatment regimen.
11 However, the use of two- and multiple-shots is not
12 ruled out, if the circumstances (or preference)
13 require. If more than one administration is required,
14 the time between administrations is preferably such as
15 to give rise to an effective anamnestic response.

16
17 The invention will now be illustrated by the following
18 examples.

19
20 EXAMPLE 1

21
22 The following examples illustrate the preparation of an
23 antigenic peptide-protein conjugate in particular a
24 GnRH based product for fertility control.

25
26
27 A Preparation of Antigen (Peptide-Protein Conjugate)

28
29 1g of GnRH modified at its carboxyl terminus from -gly
30 amide to a -gly acid is added to 1g of ovalbumin in
31 water. This is followed by the addition of a 25-fold
32 molar excess over the peptide of 1-ethyl-3-(3-dimethyl
33 aminopropyl) carbodiimide hydrochloride, giving a 0.25M

1 solution. The pH of the mixture is controlled at
2 between 6.5 and 7 by titration with 1M hydrochloric
3 acid for at least 5 hours, followed by dialysis against
4 water and then reaction in 0.5M hydroxylamine at pH 7
5 for 5 hours. The final reaction mix is dialysed
6 against water, filtered through a 0.2 micron membrane
7 and freeze dried. Progress of the reaction to form
8 peptide-protein conjugate, and dialysis to remove
9 unconjugated low molecular weight by-products is
10 monitored by analytical HPLC. The peptide content of
11 the conjugate is determined by differential amino acid
12 analysis relative to the amino acid content of carrier
13 protein alone. (The treatment with hydroxylamine helps
14 obtain a water-soluble product with consistent peptide
15 content.)

16

17

18 B Preparation of Adjuvant

19

20 30g of DEAE-dextran (eg from Pharmacia, Sweden, or
21 Sigma Chemical Co, USA) is mixed with 4.2g of saponin
22 (eg from Sigma Chemical Co, USA or as a lyophilised
23 preparation such as that sold under the trade mark
24 QUIL-A from Superfos Biosector A/S, Denmark) and 2g of
25 solid tris-(hydroxymethyl)aminomethane (eg Trizma Base
26 Sigma Chemical Co, USA). The mixture is dissolved in
27 distilled water (1.75 litres) and adjusted to pH 7 \pm
28 0.2 units with a 2M aqueous solution of Trizma (pH
29 10.5).

30

31

32

33

1 C Preparation of Antigen-Adjuvant Mixture

2 Antigen peptide-protein conjugate prepared as described
3 above, is then added to the neutralised adjuvant
4 solution and dissolved by gentle mixing at ambient
5 temperature (20°C). The solution is stirred thoroughly
6 for at least 24 hours, prior to freeze drying. The
7 dried antigen-adjuvant mix is passed through a
8 stainless steel sieve (350µm mesh) prior to tablet
9 preparation.

10

11 EXAMPLE 2

12

13 Tablet Preparation

14

15 A formulation to make a 100g powdered mixture for
16 compressing into tablets (implants) is as follows:

17

	<u>100g Batch</u>	<u>mg/tablet</u> <u>(average)</u>
21 EMCOMPRESS Calcium phosphate	72.5g	170
22 DC-Lactose	8.0g	19
23 LUBRITAB Hydrogenated		
24 vegetable oil	2.5g	6
25 Antigen/Adjuvant mix from		
26 Example 1	17.0g	40
27	—	—
28		
29 TOTAL WEIGHT : 100.0g		235mg

30

31 The batch is prepared by mixing the calcium phosphate
32 and the lactose together in a tumble mixer at 27rpm for
33 15 minutes. The antigen/adjuvant mix from Example 1 is

1 then added, and the mixture is blended together for a
 2 further 15 minutes in an ERWEKA AR400 (trade mark) cube
 3 mixer from Erweka Apparatebau GmbH, Heusenstamm, West
 4 Germany. The resulting mixture was sieved through a
 5 350 μ m mesh, and the hydrogenated vegetable oil was
 6 added to the sieved mixture and then blended for 15
 7 minutes, again in the ERWEKA AR400 cube mixer.

8
 9 The blended mixture of ingredients is compressed into
 10 tablets in a 4.5mm punch and dye, using the MANESTY SP1
 11 (trade mark) single punch tabletting machine from
 12 Manesty Machines Ltd, Liverpool, UK. The resulting
 13 tablets weighed 235mg \pm 23mg, had a diameter of 4.5mm
 14 and a length of 8.6 \pm 0.6mm.

15

16 EXAMPLE 3

17

18 The procedure of Example 1 was followed, except that
 19 the proportions of the adjuvants, buffer and antigenic
 20 conjugate were as follows:

21

22	Conjugate (GnRH-ovalbumin)	200mg
23	DEAE-dextran	6.0g
24	Trizma	400mg
25	Saponin	840mg

26

27 The DEAE-dextran, Trizma and Saponin were made up in
 28 350ml distilled water and adjusted to pH 7 with 2M
 29 Trizma. A conjugate was then added to this solution,
 30 which was thoroughly mixed for 24 hours and then freeze
 31 dried. The resulting antigen/adjuvant mix was sieved
 32 (350 μ m mesh), then mixed with the other components in
 33 the amounts given below to form implants:

1		
2	EMCOMPRESS Calcium Phosphate	30.31g
3	DC-Lactos	3.37g
4	LUBRITAB hydrogenated	
5	vegetable oil	1.04g
6	Antigen/Adjuvant Mix	6.88g
7		
8	TOTAL WEIGHT : 41.6g	
9		

10 This mixture yielded up to 175 implants weighing
11 approximately 235mg each. Each implant contained
12 approximately 1.1mg of conjugate, equivalent to about
13 125 μ g GnRH.

14

15 EXAMPLE 4

16

17 The tablets produced in Example 3 were used to
18 immunologically castrate rams (Dorset/Merino) as
19 follows.

20

21 The rams were divided into six groups, each of five
22 animals, and dosed with 1, 2 or 3 tablets in one or two
23 implantations by subcutaneous implantation by means of
24 a trocar in the neck' region below the ear.

25

26 Testicular weight at various time intervals from the
27 first implantation was measured by orchidometry, a
28 comparative palpation procedure using a graded set of
29 beads for reference. [C.M. Oldham et al Aust. J. Agric.
30 Res. 29, 173-179 (1978)]. The second implantation was
31 4 weeks after the primary implant. The results eight
32 weeks after the first implantation are shown in Figure
33 1 and demonstrate the ability of the implant

1 formulation to effect testicular atrophy in mature
2 rams.

3
4 Example 5

5
6 The implant vaccines were used to examine the effect of
7 changes in immuno-adjuvant formulation on testicular
8 development in growing ram lambs. Groups of 5 second
9 cross ram lambs 5 to 7 weeks of age were immunised
10 subcutaneously in the neck below the ear with various
11 GnRH vaccine implants having varying amounts and
12 treatments of adjuvants. The implants were made as
13 described in Example 3 except that the amounts of
14 DEAE-dextran and/or Saponin were reduced. The amounts
15 of Emcompress calcium phosphate were increased
16 accordingly to maintain implant weights at
17 approximately 235mg. The adjuvants, buffer and antigen
18 conjugates were mixed in aqueous solution for 24 hours
19 prior to freeze drying and incorporation into implants.
20 One implant was given at primary (1^o) and one at the
21 secondary (2^o) boost 5 weeks later. The results shown
22 in Table 1 illustrate the effect of varying adjuvant
23 formulation on testicular development in prepubertal
24 ram lambs. Also shown is a dry mixed antigen/adjuvant
25 formulation and a reference oil adjuvant vaccine
26 [Hoskinson et al. Aust. J. BIOTECH 4, 166-170 (1990)]
27 at 1mg antigen/2ml dose.

28
29
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Table 1

Effect of Adjuvant formulation on testicular development in ram lambs.

Group Mean Testicular weight (g).

GROUP	WEEK:0	1 ⁰	5	2 ⁰	9	13	22	Antibody titre at week 7 (1/5000cpm)
1. D1:S1 (STD)	10	25	16	17	111	7,666		
2. D1:S1 (DRY MIX)	10	68	66	102	N.T.*	6,016		
3. DO.5:S1	10	57	60	77	N.T.	7,099		
4. DO.25:S1	10	55	68	122	N.T.	5,013		
5. DO.S1	10	78	106	157	N.T.	4,580		
6. D1:SO	10	51	83	124	N.T.	4,055		
7. DO:SO	10	100	147	224	N.T.	411		
8. D1:Q	10	24	26	32	74	10,320		
9. VAX	10	25	34	20	78	10,523		
10. CONTROLS	10	108	164	249	>280	29		

CODE: D1, S1: DEAE-dextran and Saponin are in the same amounts as in Example 3.

DO, SO denotes the absence of DEAE-dextran or Saponin.

STD denotes standard formulation as in Example 3.

1 DRY MIX denotes antigen/adjuvant formulation dry mixed
2 only before implant production.

3 DO.5, DO.25: DEAE-dextran at one half and one quarter
4 respectively the amount in Example 3.

5 Q is Quil A Saponin at half the amount of Sigma Saponin
6 in Example 3 and each implant has 2 mg antigenic
7 conjugate instead of 1.1 mg.

8 VAX is the reference oil adjuvanted vaccine.

9 CONTROLS are placebo implants which contain
10 carbodiimide treated ovalbumin instead of
11 GnRH-ovalbumin conjugate.

12 N.T. denotes not tested.

13

14 Ram lambs are considered sexually competent when
15 testicular weight exceeds 120 grams (WO-A-8801177).

16 Table 1 shows that DEAE-dextran and Saponin alone or in
17 combination retard testicular development in lambs when
18 given as adjuvants in GnRH implant vaccines.

19 Combinations of the two adjuvants have a more profound
20 effect. Admixing the adjuvants and antigens in aqueous
21 solution and lyophilising the mixture results in a more
22 effective implant than simple dry admixing (compare
23 groups 1 and 2). The results demonstrate the viability
24 of solid implant vaccines in immunologically delaying
25 puberty (compare groups 1 and 8 with 10). The
26 formulation used gives comparable results to a
27 commercial oil-based liquid vaccine (compare groups 1
28 and 8 with 9).

29

30 Example 6

31 The effect of implant GnRH vaccines (single
32 administration) on testicular status in growing ram

33

1 lambs or mature rams were examined (Tabl 2 and Figure
2 2).

3
4 Groups of second cross ram lambs (3 to 5 weeks of age)
5 and mature rams (12 months) were immunised
6 subcutaneously by trocar in the neck region below the
7 ear with GnRH vaccine implants. The implants were
8 prepared as indicated for Group 8 in Example 5 (Table
9 1) in which Quil A saponin was used and each implant
10 (235mg size) contained 2 mg of GnRH conjugate. The
11 implants were used uncoated or were coated (10 μ m thick)
12 with an under layer of hydroxypropylmethylcellulose
13 ("Pharmacoat" HPMC 615; Shinetsu Chemical Co Ltd. Japan)
14 to prepare a suitable surface for the main coat (80 μ m
15 thick) of "Medisorb" 100DL lactide polymer (80-110k
16 Daltons) applied in acetone: isopropanol (70:30 w/w)
17 solvent. A protecting coat of HPMC 615 (10 μ m thick)
18 was finally applied.

19
20 The implants were pan coated using an Erweka AR 400
21 drive unit, a 9.5 litre (type DK) coating pan and an
22 Aeromatic (type Strea-1) spraying device with ER 39
23 nozzle (1.1 mm orifice).

24
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1 Table 2

2

3

4

Group mean testicular weight (g).

5

6

7

Group AWeek 0 5 7 10 15

8

9

10 Ram Lambs (n=7)

11

12	1. Q I (1° only)	14	12	19	41	80
13	2. Coated QI (1° only)	10	19	30	65	121
14	3. QI + coated QI (1° only)	10	14	21	31	61
15	4. QI (1° then 2° at week 5)	10	14	14	16	19
16	5. VAX (1° then 2° at week 5)	10	13	11	16	10
17	6. Controls	10	28	38	78	118

18

19

20

GROUP BWeek 0 4 8 12 16

21

22 Mature Rams (n=8)

23

24	1. QI (1° only)	234	208	138	144	162
25	2. Controls	244	220	222	209	210

26

27

28 CODE QI denotes an implant prepared with Quil A
 29 Saponin and 2mg antigen conjugate as in Example 5,
 30 Table 1 Group 8.

31 Coated QI denotes that the implant was subsequently
 32 coated as described in the text.

33 VAX is the reference oil adjuvant vaccine.

1 Controls are placebo implants as described in Table 2.

2

3

4 The results demonstrate that a single implantation in
5 either immature or mature rams will suppress or regress
6 testicular development. Whilst a secondary boost
7 enhances the effect, a coated implant given at the same
8 time as the first implantation allows for implants with
9 a delayed release (compare Groups A 3 and A 4).

10

11 In another group of ram lambs an uncoated implant
12 prepared according to Example 3 was given to each lamb
13 in conjunction with an implant that contained
14 cholesterol filler in various amounts in place of
15 calcium phosphate. The results are shown in Figure 2
16 and demonstrate that the use of cholesterol as an
17 additional filler (between 20% and 80% of implant
18 weight) can be used to advantage in constructing solid
19 vaccines suitable for single implantations.

20

21 EXAMPLE 7

22

23 In order to demonstrate the solid implant vaccine
24 approach for disease applications in animals we
25 undertook experiments to test serological responses to
26 a number of relevant antigens. In each case the
27 antigens were produced by Arthur Webster Pty. Ltd. (an
28 Australian veterinary vaccine manufacturer) of Sydney,
29 Australia. The example shown is a solid implant
30 vaccine for ovine footrot and is prepared from
31 concentrated purified Bacteroides nodosus pilus
32 antigens derived from recombinant Pseudomonas
33 aeruginosa representing the nine B. nodosus serogroups

A to I. All antigens were mixed together before blending into vaccine. The aqueous solution of antigen representing 100 doses was freeze dried. The dried mixture was then formulated with the following components in a manner similar to that described for Example 3.

DEAE-dextran	3.4g
Trizma	230mg
Saponin	480mg
Dried Antigen mix	100 doses
Water	200ml

The mixture was carefully stirred to dissolve the components and the pH was adjusted to 7.0 with 2M Trizma. The solution was stirred for 24 hours at 20°C prior to freeze drying. The dried antigen/adjuvant mix was sieved through a 350 μ m stainless steel mesh.

Formulations were made to contain the equivalent of either one dose (A) or about half dose (B) of antigen per implant as follows:

	A	B
EMCOMPRESS Calcium Phosphate	8.7g	9.6g
DC-Lactose	0.97g	1.07g
Lubritab	0.3g	0.3g
Antigen/Adjuvant	2.0g	1.0g

Implants were made as described in Examples 2 and 3 and administered via trocar. A single implant was used at each vaccination except where designated as "A+B" in Table 3 below - in these cases the animals were vaccinated both with one A and with one B tablet at the

1 same time at the same site. An oil adjuvanted liquid
2 vaccine in 1ml volume served as a reference standard -
3 this was prepared from the same antigen mix at the dose
4 level of the A implants.

5
6 Groups of 8 sheep were immunised with a 4 week
7 interdose interval. To illustrate the immune response,
8 individual sera were tested for response to each of 5
9 serogroups (A,B,C,D, and I); results presented below
10 (Table 3) are grand geometric means (GGM) i.e. the mean
11 of the geometric means for the 5 serogroups. The sera
12 from the sheep were tested at various intervals during
13 the trial using a normal microtitre plate agglutination
14 assay.

15

16

17 Table 3

18

19 Antibody titrations for footrot vaccines

20

21 GMM at various time intervals

22

23

24	<u>VACCINE GROUP</u>	<u>WEEK</u>	<u>0(1^o)</u>	<u>4(2^o)</u>	<u>7</u>	<u>11</u>
27	A(1 ^o)/A(2 ^o)	NT	760	4020	1440	
28	A(1 ^o)/B(2 ^o)	NT	830	4160	1350	
29	(A + B) 1 ^o only	NT	760	790	NT	
30	Standard 1 ^o , 2 ^o	NT	250	1330	770	
31	Controls	60	70	70	NT	

32

33

1 The following codes designate the vaccine treatment:

2

3 A(1^o), A(2^o): Implant A at first dose
4 /Implant A at boost.

5

6 A(1^o), B(2^o): Implant A at first dose
7 /Implant B at boost.

8

9 (A + B) 1^o only: Two implants A and B at
10 first dose, no boost dose.

11

12 Standard 1^o, 2^o: Conventional oil vaccine at
13 first dose. Conventional oil
14 vaccine at boost.

15

16 Controls: Unvaccinated sheep.

17

18 N.T.: Denotes not tested

19

20

21 The results clearly show the solid implant formulations
22 stimulate relatively higher levels of antibody
23 production than the reference oil adjuvanted vaccine,
24 provided that a second dose (boost) is given. These
25 results are particularly significant in that the
26 implants provide suitable levels of antibody in a
27 regimen commensurate with current farm management
28 practices. Implants coated with different thicknesses
29 of polymer would provide the basis of booster effects
30 from a single implantation strategy.

31

32 Similar positive results for the solid implant vaccine
33 approach were obtained with Caseous lymphadenitis

1 antigen in sheep, Botulinum in cattle and Bovine
2 Ephemeral Fever, when compared with the conventional
3 liquid vaccines currently used for these diseases.

4
5 In all implantations, whether for hormone or disease
6 vaccine, the site reactions were trivial and/or
7 non-existent and by two weeks post vaccination had
8 disappeared. In particular the presence of
9 cholesterol in formulated implants has the added
10 advantage of reducing the toxicity of the saponin and
11 may thus decrease the site reaction
12 further.

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1 CLAIMS

2

3 1. A solid vaccine composition comprising an
4 antigenic substance capable of inducing the generation
5 of antibodies on parenteral administration to an
6 animal, a saponin and a polycationic adjuvant.

7

8 2. A vaccine according to Claim 1 wherein the
9 antigenic substance gives rise to antibodies against a
10 disease causing agent.

11

12 3. A vaccine according to Claim 2 wherein the
13 disease causing agent comprises bacteria, virus, fungus
14 or protozoa.

15

16 4. A vaccine according to Claim 3 wherein the
17 disease causing agent comprises the bacteria causing
18 foot rot, botulism or caseous lymphadenitis (CLA) or
19 the viruses causing bovine ephemeral fever (BEF) or
20 foot and mouth disease.

21

22 5. A vaccine according to Claim 1 wherein the
23 antigenic substance gives rise to antibodies against an
24 agent which does not normally cause disease.

25

26 6. A vaccine according to Claim 5 wherein the agent
27 is a peptide or a non-peptide hormone.

28

29 7. A vaccine according to Claim 6 wherein the agent
30 is gonadotrophin releasing hormone (GnRH).

31

32 8. A vaccine according to Claim 6 wherein the agent
33 is growth hormone.

1

2

3 9. A vaccine according to claim 1 wher in the
4 antigenic substance comprises the entity against which
5 antibodies are to be raised.

6

7 10. A vaccine according to claim 1 wherein the
8 antigenic substance comprises a target antigenic moiety
9 conjugated to an immunogenic carrier.

10

11 11. A vaccine according to Claim 10 wherein the
12 carrier is a proteinaceous material.

13

14 12. A vaccine according to claim 1, additionally
15 including a filler.

16

17 13. A vaccine according to Claim 12 wherein the filler
18 comprises calcium phosphate.

19

20 14. A vaccine according to Claim 12 wherein the filler
21 comprises cholesterol.

22

23 15. A vaccine according to claim 1 which is formulated
24 as a powder, granules, tablets, boluses or extruded
25 strips.

26

27 16. A vaccine according to claim 15 which is adapted
28 to be implanted into a patient.

29

30 17. A vaccine according to claim 1 for fertility
31 control and immunoneutering of animals.

32

33

1 18. A vaccine composition according to claim 15 which
2 is coated with a polymer which is water impermeable but
3 erodible or is semi-permeable.

4

5 19. A vaccine composition according to claim 18
6 containing a plurality of implants, the implants having
7 coats of various thicknesses and/or erodibility
8 characteristics such that periodic delivery of the
9 antigen/adjuvant doses can be achieved.

10

11 20. An immunoadjuvant comprising a saponin and a
12 polycationic adjuvant.

13

14 21. A vaccine according to claim 1 or an
15 immunoadjuvant according to claim 20 wherein the
16 polycationic adjuvant comprises diethylaminoethyl
17 dextran (DEAE-dextran) or a salt thereof.

18

19 22. The preparation of a vaccine according to claim 1
20 by the admixing of:

21

- 22 (a) an antigenic substance;
- 23 (b) a saponin; and
- 24 (c) a polycationic adjuvant.

25

26 23. The preparation of a vaccine according to claim 22
27 comprising lyophilising a solution of:

28

- 29 (a) an antigenic substance;
- 30 (b) a saponin; and
- 31 (c) a polycationic adjuvant.

32

33

1 24. The preparation of a vaccine according to claim 23
2 wherein the solution is an aqueous solution.

3

4 25. The preparation of a vaccine according to claim 22
5 wherein an antigenic substance, a saponin and a
6 polycationic adjuvant are admixed by wet granulation
7 optionally in the presence of a filler, and the common
8 mixture is lyophilised.

9

10 26. The preparation of a vaccine according to claim 1
11 comprising coating granules of the active
12 antigen/adjuvant mix by solvent evaporation on to the
13 granules, wet granulation, or fluidised spray coating
14 or other means, with a polymer or a soluble mixture of
15 polymers, followed by the formulation into a vaccine as
16 a granulate or compressed tablets.

17

18 27. A method of treating an animal by means of
19 administering a vaccine according to claim 1.

20

21 28. The use of an antigenic substance capable of
22 inducing the generation of antibodies on parenteral
23 administration to an animal, a saponin and a
24 polycationic adjuvant in the preparation of a solid
25 vaccine composition.

26

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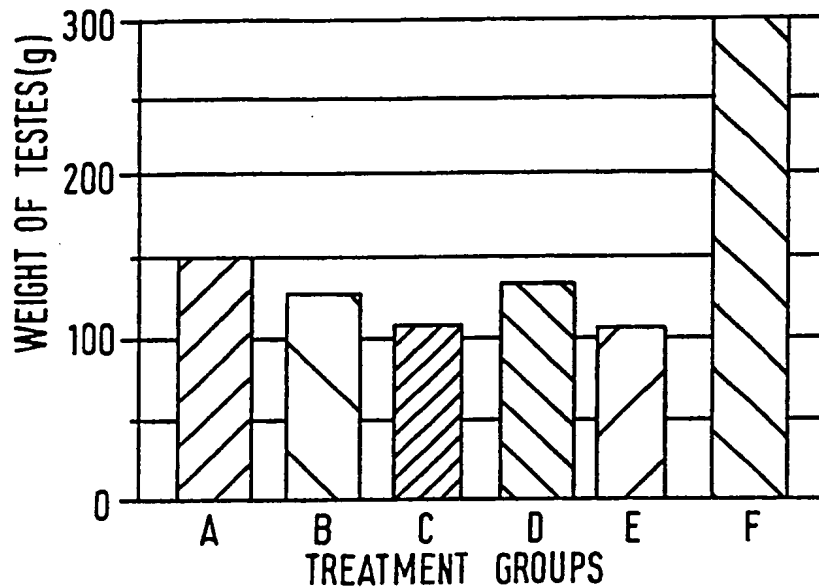
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FIG.1

Effects of GnRH Implant Vaccines on
Testicular Status in Mature Ram

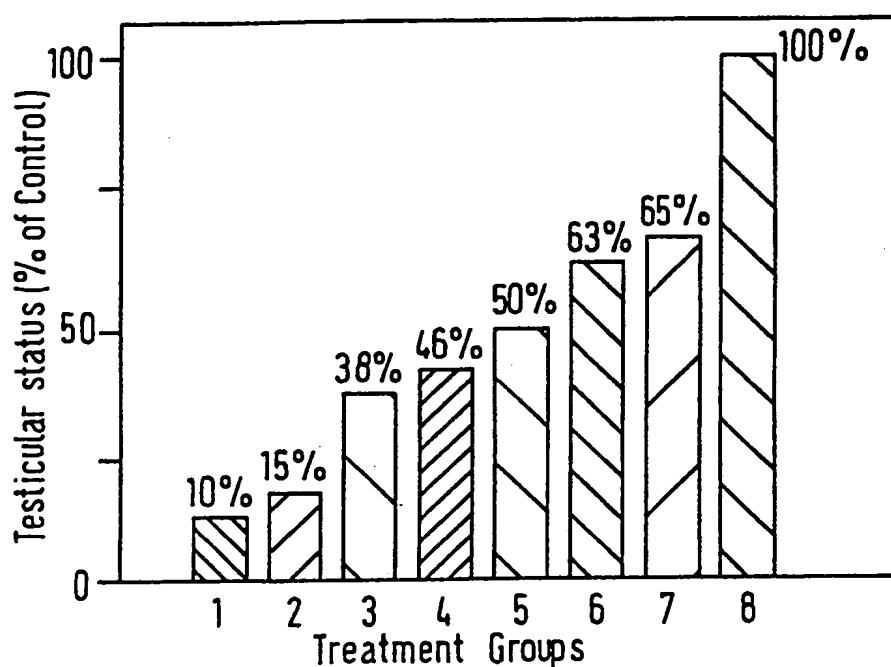


Group A 1 implant on 1 occasion
Group B 1 implant on 2 occasions
Group C 2 implants on 1 occasion
Group D 2 implants on 2 occasions
Group E 3 implants on 1 occasion
Group F Controls

2/2

FIG.2

Effects of cholesterol filler in GnRH Implant
Vaccines on Testicular Status in Growing Ram
Lambs



1. Reference of 1 adjuvant Vaccine 1° followed by 2° 4 weeks later
2. D1:S1 implant vaccine 1° followed by 2° 4 weeks later
3. D1:S1 Plus D1:S1 with 50% cholesterol filler; 1° only
4. D1:S1 Plus D1:S1 with 80% cholesterol filler; 1° only
5. D1:S1 Plus D1:S1 with 20% cholesterol filler; 1° only
6. D1:S1 Plus D1:S1 with 10% cholesterol filler; 1° only
7. D1:S1 Plus D1:S1 with no cholesterol; 1° only
8. Controls (1° only); Mean Testicular weight at week 8 is 135g

SUBSTITUTE SHEET

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: A 61 K 39/39, 39/00, 9/14, 9/20**II. FIELDS SEARCHED**Minimum Documentation Searched ⁷

Classification System

Classification Symbols

IPC⁵

A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸**III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹**

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
------------------------	--	-------------------------------------

X

EP, A, 0284406 (COOPERS ANIMAL HEALTH LTD)
28 September 1988
see page 6, lines 30-37
(cited in the application)

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20

A

WO, A, 87/06129 (DARATECH PTY. LTD)
22 October 1987
see the claims
(cited in the application)

1-26, 28

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

27th November 1990

Date of Mailing of this International Search Report

18. 12. 90

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

M. Peis

M. PEIS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 27 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1(iv) - PCT:

Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9001459
SA 40378

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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